(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 January 2001 (18.01.2001)

PCT

(10) International Publication Number
WO 01/04307 A1

(51) International Patent Classification[7]: C12N 15/12, C07K 14/47

(21) International Application Number: PCT/US00/18849

(22) International Filing Date: 11 July 2000 (11.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/353,298 13 July 1999 (13.07.1999) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/04307 A1

(54) Title: ALPHA PROTEIN - 27

(57) Abstract: The present invention relates to polynucleotide and polypeptide molecules for mammalian alpha protein-27 (Zalpha27). The polypeptides, and polynucleotides encoding them, are hormonal and may be used to regulate the functioning of the immune system. The present invention also includes antibodies to the Zalpha27 polypeptides.

ALPHA PROTEIN - 27

BACKGROUND OF THE INVENTION

The teachings of all the references cited herein are incorporated in their entirety herein by reference.

Ribonucleic acid (RNA) is often isolated in the process of discovering new genes and proteins, and making cDNA libraries. However, the isolated RNA can be destroyed if it is contaminated with ribonuclease, an enzyme that destroys RNA. Thus there is a need to discover proteins that can be used to inhibit ribonucleases.

DESCRIPTON OF THE INVENTION

The present invention addresses this need by providing for novel polypeptides and related compositions and methods that can be used to inhibit ribonucleases. Within one aspect, the present invention provides an isolated polynucleotide encoding a mammalian cytokine termed 'alpha protein-27', hereinafter referred to as "Zalpha27". Zalpha27 is defined by SEQ ID NOs 1 and 2 and has a molecular weight of about 17,054 Daltons (D).

Within a second aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding Zalpha27 polypeptide, and (c) a transcription terminator, wherein the promoter, DNA segment, and terminator are operably linked.

Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above, wherein said cell expresses a protein polypeptide encoded by the DNA segment.

Within a further aspect of the invention there is provided a chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond. The first portion of the chimeric polypeptide consists essentially of (a) a Zalpha27 polypeptide as shown in SEQ ID NO: 2 (b) allelic variants of SEQ ID NO:2; and (c) protein polypeptides that are at least 80% identical to (a) or (b). The second portion of the chimeric polypeptide consists essentially of another polypeptide such as an affinity tag. Within one embodiment the affinity tag is an immunoglobulin F_c polypeptide. The invention also provides expression vectors encoding the chimeric polypeptides and host cells transfected to produce the chimeric polypeptides.

Within an additional aspect of the invention there is provided an antibody that specifically binds to a Zalpha27 polypeptide as disclosed above, and also an anti-idiotypic antibody that neutralizes the antibody to a Zalpha27 polypeptide.

An additional embodiment of the present invention relates to a peptide or polypeptide that has the amino acid sequence of an epitope-bearing portion of a Zalpha27 polypeptide having an amino acid sequence described above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Zalpha27 polypeptide of the present invention include portions of such polypeptides with at least nine, preferably at least 15 and more preferably at least 30 to 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the present invention described above are also included in the present invention. Examples of epitope-bearing polypeptides of the present invention are SEQ ID NOs: 4 - 9. Also claimed are any of these polypeptides that are fused to another polypeptide or carrier molecule.

Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of

the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A, Nilsson *et al.*, *EMBO J. 4*:1075 (1985); Nilsson *et al.*, *Methods Enzymol. 198*:3 (1991), glutathione S transferase, Smith and Johnson, *Gene 67*:31 (1988), Glu-Glu affinity tag, Grussenmeyer *et al., Proc. Natl. Acad. Sci. USA 82*:7952-4 (1985), substance P, Flag™ peptide, Hopp *et al., Biotechnology 6*:1204-1210 (1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al., Protein Expression and Purification 2*: 95-107 (1991). DNAs encoding affinity tags are available from commercial suppliers (*e.g.*, Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

"Angiogenic" denotes the ability of a compound to stimulate the formation of new blood vessels from existing vessels, acting alone or in concert with one or more additional compounds. Angiogenic activity is measurable as endothelial

cell activation, stimulation of protease secretion by endothelial cells, endothelial cell migration, capillary sprout formation, and endothelial cell proliferation.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of $<10^9$ M^{-1}.

The term "complements of a polynucleotide molecule" is a polynucleotide molecule having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to "overlap" a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example, representative contigs to the polynucleotide sequence 5'-ATGGCTTAGCTT-3' are 5'-TAGCTTgagtct-3' and 3'-gtcgacTACCGA-5'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, *Nature 316*:774-78 (1985).

An "isolated" polypeptide or protein is a polypeptide or protein that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. When used in this context, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The term "operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended

purposes, *e.g.*, transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, a-globin, b-globin, and myoglobin are paralogs of each other.

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will in general not exceed 20 nt in length.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of

RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.*, thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.*, PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in

which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (*e.g.*, gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

The present invention provides novel cytokine polypeptides/proteins. The novel cytokine, termed "alpha helical protein-32" hereinafter referred to as "Zalpha27" was discovered and identified to be a cytokine by the presence of polypeptide and polynucleotide features characteristic of four-helix-bundle cytokines (*e.g.*, erythropoietin, thrombopoietin, G-CSF, IL-2, IL-4, leptin and growth hormone). Analysis of the amino acid sequence shown in SEQ ID NO:2 indicates a signal sequence which extends from the methionine at position 1 to and including amino acid residue 25. Thus the mature sequence extends from amino acid residue 26, a glutamine, to an including amino acid residue 170, a phenylalanine. The mature Zalpha27 polypeptide is also represented by the amino acid sequence of SEQ ID NO:3 which has an unglycosylated molecular weight of approximately 16,578 Daltons (D).

POLYNUCLEOTIDES:

The present invention also provides polynucleotide molecules, including DNA and RNA molecules, that encode the Zalpha27 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules.

Polynucleotides, generally a cDNA sequence, of the present invention encode the described polypeptides herein. A cDNA sequence which encodes a polypeptide of the present invention is comprised of a series of codons, each amino acid residue of the polypeptide being encoded by a codon and each codon being comprised of three nucleotides. The amino acid residues are encoded by their respective codons as follows.

Alanine (Ala) is encoded by GCA, GCC, GCG or GCT;
Cysteine (Cys) is encoded by TGC or TGT;
Aspartic acid (Asp) is encoded by GAC or GAT;
Glutamic acid (Glu) is encoded by GAA or GAG;
Phenylalanine (Phe) is encoded by TTC or TTT;
Glycine (Gly) is encoded by GGA, GGC, GGG or GGT;
Histidine (His) is encoded by CAC or CAT;
Isoleucine (Ile) is encoded by ATA, ATC or ATT;
Lysine (Lys) is encoded by AAA, or AAG;
Leucine (Leu) is encoded by TTA, TTG, CTA, CTC, CTG or CTT;
Methionine (Met) is encoded by ATG;
Asparagine (Asn) is encoded by AAC or AAT;
Proline (Pro) is encoded by CCA, CCC, CCG or CCT;
Glutamine (Gln) is encoded by CAA or CAG;
Arginine (Arg) is encoded by AGA, AGG, CGA, CGC, CGG or CGT;
Serine (Ser) is encoded by AGC, AGT, TCA, TCC, TCG or TCT;

Threonine (Thr) is encoded by ACA, ACC, ACG or ACT;
Valine (Val) is encoded by GTA, GTC, GTG or GTT;
Tryptophan (Trp) is encoded by TGG; and
Tyrosine (Tyr) is encoded by TAC or TAT.

It is to be recognized that according to the present invention, when a polynucleotide is claimed as described herein, it is understood that what is claimed are both the sense strand, the anti-sense strand, and the DNA as double-stranded having both the sense and anti-sense strand annealed together by their respective hydrogen bonds. Also claimed is the messenger RNA (mRNA) that encodes the polypeptides of the president invention, and which mRNA is encoded by the cDNA described herein. Messenger RNA (mRNA) will encode a polypeptide using the same codons as those defined herein, with the exception that each thymine nucleotide (T) is replaced by a uracil nucleotide (U).

One of ordinary skill in the art will also appreciate that different species can exhibit "preferential codon usage." In general, see, Grantham, *et al.*, *Nuc. Acids Res. 8*:1893-1912 (1980); Haas, *et al. Curr. Biol. 6*:315-324 (1996); Wain-Hobson, *et al.*, *Gene 13*:355-364 (1981); Grosjean and Fiers, *Gene 18*:199-209 (1982); Holm, *Nuc. Acids Res. 14*:3075-3087 (1986); Ikemura, *J. Mol. Biol. 158*:573-597 (1982). As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid Threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Sequences

containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is up to about 0.03 M at pH 7 and the temperature is at least about 60°C.

As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for preparing DNA and RNA are well known in the art. In general, RNA is isolated from a tissue or cell that produces large amounts of Zalpha27 RNA. Such tissues and cells are identified by Northern blotting, Thomas, *Proc. Natl. Acad. Sci. USA 77*:5201 (1980) and are discussed below. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient, Chirgwin *et al.*, *Biochemistry 18*:52-94 (1979). Poly $(A)^+$ RNA is prepared from total RNA using the method of Aviv and Leder, *Proc. Natl. Acad. Sci. USA 69*:1408-1412 (1972). Complementary DNA (cDNA) is prepared from poly$(A)^+$ RNA using known methods. In the alternative, genomic DNA can be isolated. Polynucleotides encoding Zalpha27 polypeptides are then identified and isolated by, for example, hybridization or PCR.

A full-length clone encoding Zalpha27 can be obtained by conventional cloning procedures. Complementary DNA (cDNA) clones are preferred, although for some applications (*e.g.*, expression in transgenic animals) it may be preferable to use a genomic clone, or to modify a cDNA clone to include at least one genomic intron. Methods for preparing cDNA and genomic clones are well known and within the level of ordinary skill in the art, and include the use of the sequence disclosed herein, or parts

thereof, for probing or priming a library. Expression libraries can be probed with antibodies to Zalpha27, receptor fragments, or other specific binding partners.

The polynucleotides of the present invention can also be synthesized using DNA synthesizers. Currently the method of choice is the phosphoramidite method. If chemically synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 bp) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 bp), however, special strategies must be invoked, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. See Glick and Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA,* (ASM Press, Washington, D.C. 1994); Itakura *et al., Annu. Rev. Biochem. 53*: 323-356 (1984) and Climie *et al., Proc. Natl. Acad. Sci. USA 87*:633-637 (1990).

The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are Zalpha27 polypeptides from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of human Zalpha27 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses Zalpha27 as disclosed herein. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A Zalpha27-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with

one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No. 4,683,202), using primers designed from the representative human Zalpha27 sequence disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to Zalpha27 polypeptide. Similar techniques can also be applied to the isolation of genomic clones.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of human Zalpha27 and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the Zalpha27 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention also provides isolated Zalpha27 polypeptides that are substantially homologous to the polypeptides of SEQ ID NO:2 and their orthologs. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2 or their orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2 or its orthologs.) Percent sequence identity is determined by conventional methods. See, for example, Altschul *et al.*, *Bull. Math. Bio. 48*: 603-616 (1986) and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915-10919 (1992). Briefly, two amino acid

sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 1 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

Table 1

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Those skilled in the art appreciate that there are many established algorithms to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence and the amino acid sequence of a putative variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions or deletions. The ten regions with the highest density of identities are then re-scored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from four to six.

The present invention includes nucleic acid molecules that encode a polypeptide having one or more conservative amino acid changes, compared with the

amino acid sequence of SEQ ID NO:2. The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins [Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA 89*:10915 (1992)]. Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. As used herein, the language "conservative amino acid substitution" refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0,1,2, or 3. Preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (*e.g.*, 1,2 or 3), while more preferred conservative substitutions are characterized by a BLOSUM62 value of at least 2 (*e.g.*, 2 or 3). Accordingly the present invention claims those polypeptides which are at least 90%, preferably 95% and most preferably 99% identical to SEQ ID NO:3 and which are able to stimulate antibody production in a mammal, and said antibodies are able to bind the native sequence of SEQ ID NO:2.

Variant Zalpha27 polypeptides or substantially homologous Zalpha27 polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 2) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag. The present invention thus includes polypeptides of from 20 to 30 amino acid residues that comprise a sequence that is at least 90%, preferably at least 95%, and more preferably 99% or more identical to the corresponding region of SEQ ID NO:4. Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the Zalpha27 polypeptide and the affinity tag. Preferred such sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 2

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

The present invention further provides a variety of other polypeptide fusions [and related multimeric proteins comprising one or more polypeptide fusions]. For example, a Zalpha27 polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin-Zalpha27 polypeptide fusions can be expressed in genetically engineered cells [to produce a variety of multimeric Zalpha27 analogs]. Auxiliary domains can be fused to Zalpha27 polypeptides to target them to specific cells, tissues, or macromolecules (*e.g.*, collagen). For example, a Zalpha27 polypeptide or protein could be targeted to a predetermined cell type by fusing a Zalpha27 polypeptide to a ligand that specifically binds to a receptor on the surface of the target cell. In this way, polypeptides and proteins can be targeted for therapeutic or diagnostic purposes. A

Zalpha27 polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan *et al.*, *Connective Tissue Research 34*:1-9 (1996).

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs.

Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson *et al.*, *J. Am. Chem. Soc. 113*:2722 (1991); Ellman *et al.*, *Methods Enzymol. 202*:301 (1991; Chung *et al.*, *Science 259*:806-809 (1993); and Chung *et al.*, *Proc. Natl. Acad. Sci. USA 90*:10145-1019 (1993). In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs, Turcatti *et al.*, *J. Biol. Chem. 271*:19991-19998 (1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its

natural counterpart. See, Koide *et al.*, *Biochem. 33*:7470-7476 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions, Wynn and Richards, *Protein Sci. 2*:395-403 (1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Zalpha27 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis, Cunningham and Wells, *Science 244*: 1081-1085 (1989); Bass *et al.*, *Proc. Natl. Acad. Sci. USA 88*:4498-502 (1991). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, *J. Biol. Chem. 271*:4699-708, 1996. Sites of ligand-receptor interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al.*, *Science 255*:306-312 (1992); Smith *et al.*, *J. Mol. Biol. 224*:899-904 (1992); Wlodaver *et al.*, *FEBS Lett. 309*:59-64 (1992).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer, *Science 241*:53-57 (1988) or Bowie and Sauer, *Proc. Natl. Acad. Sci. USA 86*:2152-2156 (1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used

include phage display, *e.g.*, Lowman *et al.*, *Biochem. 30*:10832-10837 (1991); Ladner *et al.*, U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis, Derbyshire *et al.*, *Gene 46*:145 (1986); Ner *et al.*, *DNA 7*:127 (1988).

Variants of the disclosed Zalpha27 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, *Nature 370*:389-391, (1994), Stemmer, *Proc. Natl. Acad. Sci. USA 91*:10747-10751 (1994) and WIPO Publication WO 97/20078. Briefly, variant DNAs are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed herein, one of ordinary skill in the art can identify and/or prepare a variety of polypeptide fragments or variants of SEQ ID NO:2 or that retain the properties of the wild-type Zalpha27 protein. For any Zalpha27 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above.

PROTEIN PRODUCTION

The Zalpha27 polypeptides of the present invention, including full-length polypeptides, biologically active fragments, and fusion polypeptides, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook *et al.*, Molecular Cloning: *A Laboratory Manual*, 2nd ed., (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc., NY, 1987).

In general, a DNA sequence encoding a Zalpha27 polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a Zalpha27 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of Zalpha27, or may be derived from another secreted protein (*e.g.*, t-PA) or synthesized *de novo*. The secretory signal sequence is operably linked to the Zalpha27

DNA sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the DNA sequence of interest (see, *e.g.*, Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830).

Alternatively, the secretory signal sequence contained in the polypeptides of the present invention is used to direct other polypeptides into the secretory pathway. The present invention provides for such fusion polypeptides. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein, such as a receptor. Such fusions may be used *in vivo* or *in vitro* to direct peptides through the secretory pathway.

Cultured mammalian cells are suitable hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection, Wigler *et al.*, *Cell 14*:725 (1978); Corsaro and Pearson, *Somatic Cell Genetics 7*:603 (1981); Graham and Van der Eb, *Virology 52*:456 (1973), electroporation, Neumann *et al.*, *EMBO J. 1*:841-845 (1982), DEAE-dextran mediated transfection (Ausubel *et al.*, *ibid.*, and liposome-mediated transfection, Hawley-Nelson *et al.*, *Focus 15*:73 (1993); Ciccarone *et al.*, *Focus 15*:80 (1993), and viral vectors, Miller and Rosman, *BioTechniques 7*:980(1989); Wang and Finer, *Nature Med. 2*:714 (1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK

(ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham *et al.*, *J. Gen. Virol. 36*:59 (1977) and Chinese hamster ovary (*e.g.* CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, *e.g.*, U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (*e.g.* hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar *et al.*, *J. Biosci.*

(Bangalore) 11:47 (1987). Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino *et al.*, U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463. Insect cells can be infected with recombinant baculovirus, commonly derived from Autographa californica nuclear polyhedrosis virus (AcNPV). DNA encoding the Zalpha27 polypeptide is inserted into the baculoviral genome in place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of homologous DNA recombination between wild-type AcNPV and a transfer vector containing the Zalpha27 flanked by AcNPV sequences. Suitable insect cells, *e.g.* SF9 cells, are infected with wild-type AcNPV and transfected with a transfer vector comprising a Zalpha27 polynucleotide operably linked to an AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King, L.A. and Possee, R.D., *The Baculovirus Expression System: A Laboratory Guide*, (Chapman & Hall, London); O'Reilly, D.R. *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press, New York, New York, 1994); and, Richardson, C. D., Ed., *Baculovirus Expression Protocols. Methods in Molecular Biology*, (Humana Press, Totowa, NJ 1995). Natural recombination within an insect cell will result in a recombinant baculovirus which contains Zalpha27 driven by the polyhedrin promoter. Recombinant viral stocks are made by methods commonly used in the art.

The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow, V.A, *et al.*, *J Virol 67*:4566 (1993). This system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Zalpha27 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case Zalpha27. However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as P*cor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous

for expressing secreted proteins. See, Hill-Perkins, M.S. and Possee, R.D., *J Gen Virol 71*:971 (1990); Bonning, B.C. *et al.*, *J Gen Virol 75*:1551 (1994); and, Chazenbalk, G.D., and Rapoport, B., *J Biol Chem 270*:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed that replace the native Zalpha27 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native Zalpha27 secretory signal sequence. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Zalpha27 polypeptide, for example, a Glu-Glu epitope tag, Grussenmeyer, T. *et al.*, *Proc Natl Acad Sci. 82*:7952 (1985). Using a technique known in the art, a transfer vector containing Zalpha27 is transformed into *E. coli*, and screened for bacmids that contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, *e.g.* Sf9 cells. Recombinant virus that expresses Zalpha27 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall army worm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA* (ASM Press, Washington, D.C., 1994). Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent #5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of

0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the recombinant Zalpha27 polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing the Zalpha27 polypeptide is filtered through micropore filters, usually 0.45 μm pore size. Procedures used are generally described in available laboratory manuals (King, L. A. and Possee, R.D., *ibid.*; O'Reilly, D.R. *et al.*, *ibid.*; Richardson, C. D., *ibid.*). Subsequent purification of the Zalpha27 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). A preferred vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al., J. Gen. Microbiol. 132*:3459 (1986) and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be

utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention.

Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art; see, *e.g.*, Sambrook *et al.*, *ibid.*). When expressing a Zalpha27 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient, which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of about 25°C to 35°C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking of small flasks or sparging of fermentors. A preferred culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, MI), 1% Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and 0.006% L-leucine).

Another embodiment of the present invention provides for a peptide or polypeptide comprising an epitope-bearing portion of a Zalpha27 polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. A region of a protein to which an antibody can bind is defined as an "antigenic epitope". See for instance, Geysen, H.M. *et al.*, *Proc. Natl. Acad Sci. USA 81*:3998-4002 (1984). As to the selection of peptides or polypeptides bearing an antigenic epitope (*i.e.*, that contain a region of a protein molecule to which an antibody can bind), it is well known in the art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See Sutcliffe, J.G. *et al. Science 219*:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (*i.e.*, immunogenic epitopes) nor to the amino or carboxyl terminals. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer soluble peptides, especially those containing proline residues, usually are effective Examples of epitope-bearing polypeptides of the present invention are the following polypeptides: a polypeptide containing the sequence extending from amino acid residue 8, a proline, through amino acid residue 68, and arginine of SEQ ID NO: 2, also represented by SEQ ID NO:4; a polypeptide containing the sequence extending from amino acid residue 92, an arginine, to and including amino acid residue 129, a glutamic acid of SEQ ID NO:2, also represented by SEQ ID NO: 5; a polypeptide containing the sequence extending from amino acid residue 151, an asparagine, to and including amino acid residue 269, an alanine of SEQ ID NO:2, also represented by SEQ ID NO:6; a polypeptide containing the sequence extending from amino acid residue 289, a threonine, to and including amino acid residue 454, a glutamic acid of SEQ ID NO:2, also defined by SEQ ID NO:7; a polypeptide containing the sequence extending from amino acid residue 482, a valine, to and including amino acid residue 618, a glutamic acid of SEQ ID NO:2, also defined by SEQ ID NO:8; a polypeptide containing the sequence

extending from amino acid residue 873, an asparagine, to and including amino acid residue 975, a serine of SEQ ID NO:2, also defined by SEQ ID NO:9.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies that bind specifically to a polypeptide of the invention. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine, preferably between 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that react with the protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues and hydrophobic residues are preferably avoided); and sequences containing proline residues are particularly preferred. All of the polypeptides shown in the sequence listing contain antigenic epitopes to be used according to the present invention, however, specifically designed antigenic epitopes include the peptides defined by SEQ ID NOs: 4-9. The present invention also provides polypeptide fragments or peptides comprising an epitope-bearing portion of a Zalpha1 polypeptide described herein. Such fragments or peptides may comprise an "immunogenic epitope," which is a part of a protein that elicits an antibody response when the entire protein is used as an immunogen. Immunogenic epitope-bearing peptides can be identified using standard methods [see, for example, Geysen *et al., supra.* See also U.S. Patent No. 4,708,781 (1987) further describes how to identify a peptide bearing an immunogenic epitope of a desired protein.

Protein Isolation

It is preferred to purify the polypeptides of the present invention to ≥80% purity, more preferably to ≥90% purity, even more preferably ≥95% purity, and

particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Expressed recombinant Zalpha27 polypeptides (or chimeric Zalpha27 polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988).

The polypeptides of the present invention can be isolated by exploitation of their properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate, Sulkowski, *Trends in Biochem.* 3:1 (1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography. *Methods in Enzymol.*, Vol. *182*, "Guide to Protein Purification", M. Deutscher, (ed.),page 529-539 (Acad. Press, San Diego, 1990). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (*e.g.*, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Moreover, using methods described in the art, polypeptide fusions, or hybrid Zalpha27 proteins, are constructed using regions or domains of the inventive Zalpha27, Sambrook *et al.*, *ibid.*, Altschul *et al.*, *ibid.*, Picard, *Cur. Opin. Biology*, 5:511 (1994). These methods allow the determination of the biological importance of larger domains or regions in a polypeptide of interest. Such hybrids may alter reaction kinetics, binding, constrict or expand the substrate specificity, or alter tissue and cellular localization of a polypeptide, and can be applied to polypeptides of unknown structure.

Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. For example, part or all of a domain(s) conferring a biological function may be swapped between Zalpha27 of the present invention with the functionally equivalent domain(s) from another family member. Such domains include, but are not limited to, the secretory signal sequence, conserved, and significant

domains or regions in this family. Such fusion proteins would be expected to have a biological functional profile that is the same or similar to polypeptides of the present invention or other known family proteins, depending on the fusion constructed. Moreover, such fusion proteins may exhibit other properties as disclosed herein.

Antagonists

Antagonists are useful as research reagents for characterizing sites of ligand-receptor interaction. Inhibitors of Zalpha27 activity (Zalpha27 antagonists) include anti-Zalpha27 antibodies and soluble Zalpha27 receptors, as well as other peptidic and non-peptidic agents (including ribozymes).

Zalpha27 can also be used to identify inhibitors (antagonists) of its activity. Test compounds are added to the assays disclosed herein to identify compounds that inhibit the activity of Zalpha27. In addition to those assays disclosed herein, samples can be tested for inhibition of Zalpha27 activity within a variety of assays designed to measure receptor binding or the stimulation/inhibition of Zalpha27-dependent cellular responses. For example, Zalpha27-responsive cell lines can be transfected with a reporter gene construct that is responsive to a Zalpha27-stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and will generally comprise a Zalpha27-DNA response element operably linked to a gene encoding a protein that can be assayed, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE), Nasrin *et al.*, *Proc. Natl. Acad. Sci. USA 87*:5273 (1990) and serum response elements (SRE) (Shaw *et al. Cell 56*: 563 (1989). Cyclic AMP response elements are reviewed in Roestler *et al.*, *J. Biol. Chem. 263* (19):9063 (1988) and Habener, *Molec. Endocrinol. 4* (8):1087 (1990). Hormone response elements are reviewed in Beato, *Cell 56*:335 (1989). Candidate compounds, solutions, mixtures or extracts are tested for the ability to inhibit the activity of Zalpha27 on the target cells as evidenced by a decrease in Zalpha27 stimulation of reporter gene expression. Assays of this type will detect compounds that

directly block Zalpha27 binding to cell-surface receptors, as well as compounds that block processes in the cellular pathway subsequent to receptor-ligand binding. In the alternative, compounds or other samples can be tested for direct blocking of Zalpha27 binding to receptor using Zalpha27 tagged with a detectable label (*e.g.*, ^{125}I, biotin, horseradish peroxidase, FITC, and the like). Within assays of this type, the ability of a test sample to inhibit the binding of labeled Zalpha27 to the receptor is indicative of inhibitory activity, which can be confirmed through secondary assays. Receptors used within binding assays may be cellular receptors or isolated, immobilized receptors.

A Zalpha27 polypeptide can be expressed as a fusion with an immunoglobulin heavy chain constant region, typically an F_C fragment, which contains two constant region domains and lacks the variable region. Methods for preparing such fusions are disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two non-Ig polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used to affinity purify the ligand. For use in assays, the chimeras are bound to a support via the F_C region and used in an ELISA format.

A Zalpha27 ligand-binding polypeptide can also be used for purification of ligand. The polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/ anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, *J. Immunol. Methods 145*:229 (1991) and Cunningham and Wells, *J. Mol. Biol. 234*:554 (1993). A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity, Scatchard, *Ann. NY Acad. Sci. 51*: 660 (1949) and calorimetric assays, Cunningham *et al., Science 253*:545 (1991); Cunningham *et al., Science 245*:821 (1991).

Zalpha27 polypeptides can also be used to prepare antibodies that specifically bind to Zalpha27 epitopes, peptides or polypeptides. The Zalpha27 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. Suitable antigens would be the Zalpha27 polypeptides encoded by SEQ ID NOs: 4 - 9. Antibodies generated from this immune response can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, *Current Protocols in Immunology*, Cooligan, *et al.* (eds.), National Institutes

of Health, (John Wiley and Sons, Inc., 1995); Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition* (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications* (CRC Press, Inc., Boca Raton, FL, 1982).

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a Zalpha27 polypeptide or a fragment thereof. The immunogenicity of a Zalpha27 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Zalpha27 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced.

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to Zalpha27 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled Zalpha27 protein or peptide). Genes encoding polypeptides having potential Zalpha27 polypeptide-binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides that interact with a known target, which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner *et al.*, US Patent NO. 5,223,409; Ladner *et al.*, US Patent NO. 4,946,778; Ladner *et al.*, US Patent NO. 5,403,484 and Ladner *et al.*, US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Zalpha27 sequences disclosed herein to identify proteins which bind to Zalpha27. These "binding proteins" which interact with Zalpha27 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble polypeptides as marker of underlying pathology or disease. These binding proteins can also act as Zalpha27 "antagonists" to block Zalpha27 binding and signal transduction *in vitro* and *in vivo*.

Antibodies are determined to be specifically binding if: 1. they exhibit a threshold level of binding activity, and 2. they do not significantly cross-react with related polypeptide molecules. First, antibodies herein specifically bind if they bind to a Zalpha27 polypeptide, peptide or epitope with a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis.

Second, antibodies are determined to specifically bind if they do not significantly cross-react with related polypeptides. Antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect Zalpha27 but not known related polypeptides using a standard Western blot analysis (Ausubel *et al.*, *ibid.*). Examples of known related polypeptides are orthologs, proteins from the same species that are members of a protein family (*e.g.* IL-16), Zalpha27 polypeptides, and non-human Zalpha27. Moreover, antibodies may be "screened against" known related polypeptides to isolate a population that specifically binds to the inventive polypeptides. For example, antibodies raised to Zalpha27 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to Zalpha27 will flow through the matrix under the proper buffer conditions. Such screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to closely related polypeptides, *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.) (Cold Spring Harbor Laboratory Press, 1988); *Current Protocols in Immunology*, Cooligan, *et al.* (eds.), National Institutes of Health (John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art. See, *Fundamental Immunology*, Paul (eds.) (Raven Press, 1993); Getzoff *et al.*, *Adv. in Immunol. 43*: 1-98 (1988); *Monoclonal Antibodies: Principles and Practice*, Goding, J.W. (eds.), (Academic Press Ltd., 1996); Benjamin *et al.*, *Ann. Rev. Immunol. 2*: 67-101 (1984).

A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Zalpha27 proteins or peptides. Exemplary

assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.) (Cold Spring Harbor Laboratory Press, 1988). Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant Zalpha27 protein or polypeptide.

Antibodies to Zalpha27 may be used for tagging cells that express Zalpha27 and for isolating Zalpha27 by affinity purification.

Use of Zalpha27

It has been discovered that Zalpha27 can inhibit the digestion of RNA by ribonucleases. The ideal concentration of Zalpha27 should be about 100 µM in solution to inhibit the action of ribonucleases.

EDUCATIONAL KIT UTILITY OF ZALPHA27 POLYPEPTIDES, POLYNUCLEOTIDES AND ANTIBODIES

Polynucleotides and polypeptides of the present invention will additionally find use as educational tools as a laboratory practicum kits for courses related to genetics and molecular biology, protein chemistry and antibody production and analysis. Due to its unique polynucleotide and polypeptide sequence molecules of Zalpha27 can be used as standards or as "unknowns" for testing purposes. For example, Zalpha27 polynucleotides can be used as an aid, such as, for example, to teach a student how to prepare expression constructs for bacterial, viral, and/or mammalian expression, including fusion constructs, wherein *Zalpha27* is the gene to be expressed; for determining the restriction endonuclease cleavage sites of the polynucleotides; determining mRNA and DNA localization of *Zalpha27* polynucleotides in tissues (i.e., by Northern and Southern blotting as well as polymerase chain reaction); and for identifying related polynucleotides and polypeptides by nucleic acid hybridization.

Zalpha27 polypeptides can be used educationally as an aid to teach preparation of antibodies; identifying proteins by Western blotting; protein purification; determining the weight of expressed Zalpha27 polypeptides as a ratio to total protein expressed; identifying peptide cleavage sites; coupling amino and carboxyl terminal tags; amino acid sequence analysis, as well as, but not limited to monitoring biological activities of both the native and tagged protein (i.e., receptor binding, signal transduction, proliferation, and differentiation) *in vitro* and *in vivo*. Zalpha27 polypeptides can also be used to teach analytical skills such as mass spectrometry, circular dichroism to determine conformation, in particular the locations of the disulfide bonds, x-ray crystallography to determine the three-dimensional structure in atomic detail, nuclear magnetic resonance spectroscopy to reveal the structure of proteins in solution. For example, a kit containing the Zalpha27 can be given to the student to analyze. Since the amino acid sequence would be known by the professor, the protein can be given to the student as a test to determine the skills or develop the skills of the student, the teacher would then know whether or not the student has correctly analyzed the polypeptide. Since every polypeptide is unique, the educational utility of Zalpha27 would be unique unto itself.

The antibodies which bind specifically to Zalpha27 can be used as a teaching aid to instruct students how to prepare affinity chromatography columns to purify Zalpha27, cloning and sequencing the polynucleotide that encodes an antibody and thus as a practicum for teaching a student how to design humanized antibodies. The Zalpha27 gene, polypeptide or antibody would then be packaged by reagent companies and sold to universities so that the students gain skill in art of molecular biology. Because each gene and protein is unique, each gene and protein creates unique challenges and learning experiences for students in a lab practicum. Such educational kits containing the *Zalpha27* gene, polypeptide, or antibody, are considered within the scope of the present invention.

The invention is further illustrated by the following non-limiting examples.

Example 1

Hematopoietic Cell cDNA Library

cDNAs from human hematopoietic cell lines, K562 (ATCC #CCL243), Daudi (ATCC #CCL213, HL-60(ATCC CCL240), MOLT-4 (ATCC #CRL1582) and Raji ATCC #CCL86 were synthesized in separate reactions and size fractionated in the following manner. RNA extracted from each one of the cell lines was reversed transcribed in the following manner. The first strand cDNA reaction contained 10 µl of twice poly d(T)-selected poly $(A)^+$ mRNA from 562, Daudi, HL-60, MOLT-4 or Raji Cells (Clontech, Palo Alto, CA) at a concentration of 1.0 mg/ml, and 2 µl of 20 pmole/µl first strand primer SEQ ID NO:10 (GTC TGG GTT CGC TAC TCG AGG CGG CCG CTA TTT TTT TTT TTT TTT TTT) containing an *Xho* I restriction site. The mixture was heated at 70°C for 3.0 minutes and cooled by chilling on ice. First strand cDNA synthesis was initiated by the addition of 8 µl of first strand buffer (5x SUPERSCRIPT™ buffer; Life Technologies, Gaithersburg, MD), 4.0 µl of 100 mM dithiothreitol, and 3.0 µl of a deoxynucleotide triphosphate (dNTP) solution containing 10 mM each of dTTP, dATP, dGTP and 5-methyl-dCTP (Pharmacia LKB Biotechnology, Piscataway, NJ) to the RNA-primer mixture. The reaction mixture was incubated at 37° C for 2 minutes, followed by the addition of 10 µl of 200 U/µl RNase H⁻ reverse transcriptase (SUPERSCRIPT II®; Life Technologies). The efficiency of the first strand synthesis was analyzed in a parallel reaction by the addition of 10 µCi of ^{32}P-αdCTP to a 5 µl aliquot from one of the reaction mixtures to label the reaction for analysis. The reactions were incubated at 37°C for 10 minutes, 45°C for 50 minutes, then incubated at 50°C for 10 minutes. Unincorporated ^{32}P-αdCTP in the labeled reaction was removed by chromatography on a 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA). The unincorporated nucleotides and primers in

the unlabeled first strand reactions were removed by chromatography on 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA). The length of labeled first strand cDNA was determined by agarose gel electrophoresis.

The second strand reaction contained 135 µl of the unlabeled first strand cDNA, 40 µl of 5x polymerase I buffer (125 mM Tris: HCl, pH 7.5, 500 mM KCl, 25 mM $MgCl_2$, 50mM $(NH_4)_2SO_4$)), 2.5 µl of 100 mM dithiothreitol, 5.0 µl of a solution containing 10 mM of each deoxynucleotide triphosphate, 7 µl of 5 mM β-NAD, 2.5 µl of 10 U/µl *E. coli* DNA ligase (New England Biolabs; Beverly, MA), 7 µl of 10 U/µl *E. coli* DNA polymerase I (New England Biolabs, Beverly, MA), and 2.0 µl of 2 U/µl RNase H (Life Technologies, Gaithersburg, MD). A 10 µl aliquot from one of the second strand synthesis reactions was labeled by the addition of 10 µCi ^{32}P-αdCTP to monitor the efficiency of second strand synthesis. The reactions were incubated at 16° C for two hours, followed by the addition of 1 µl of a 10 mM dNTP solution and 5.0 µl T4 DNA polymerase (10 U/µl, Boehringer Mannheim, Indianapolis, IN) and incubated for an additional 10 minutes at 16°C. Unincorporated ^{32}P-αdCTP in the labeled reaction was removed by chromatography through a 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA) before analysis by agarose gel electrophoresis. The reaction was terminated by the addition of 20.0 µl 0.5 M EDTA and extraction with phenol/chloroform and chloroform followed by ethanol precipitation in the presence of 3.0 M Na acetate and 2 µl of PELLET PAINT® carrier (Novagen, Madison, WI). The cDNAs were ethanol precipitated a second time to remove possible trace levels of EDTA. The yield of cDNA was estimated to be approximately 2 µg from starting mRNA template of 10 µg.

Eco RI adapters were ligated onto the 5' ends of the cDNA described above to enable cloning into an expression vector. A 12.0 µl aliquot of cDNA (~2.0 µg) and 4 µl of 69 pmole/µl of *Eco* RI adapter (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) were mixed with 2.5 µl 10x ligase buffer (660 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$), 2.5 µl of 10 mM ATP, 3.0 µl 0.1 M DTT and 1 µl of 15 U/µl T4

DNA ligase (Promega Corp., Madison, WI). The reaction was incubated in a 0° to 22° C temperature gradient for 48 hours. The reaction was terminated by the adding 65 µl H_2O and 10 µl 10X H buffer (Boehringer Mannheim, Indianapolis, IN) and incubating the mixture at 70° C for 20 minutes.

To facilitate the directional cloning of the cDNA into an expression vector, the cDNA was digested with *Xho* I, resulting in a cDNA having a 5' *Eco* RI cohesive end and a 3' *Xho* I cohesive end. The *Xho* I restriction site at the 3' end of the cDNA had been previously introduced. Restriction enzyme digestion was carried out in a reaction mixture by the addition of 1.0 µl of 40 U/µl *Xho* I (Boehringer Mannheim, Indianapolis, IN). Digestion was carried out at 37°C for one hour. The reaction was terminated by incubation at 70°C for 20 minutes and chromatography through a 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA).

The cDNA was ethanol precipitated, washed with 70% ethanol, air dried and resuspended in 13.5 µl water, 2 µl of 10X kinase buffer (660 mM Tris-HCl, pH 7.5, 100 mM $MgCl_2$), 0.5 µl 0.1 M DTT, 3 µl 10 mM ATP, 1.0 µl T4 polynucleotide kinase (10 U/µl, Life Technologies, Gaithersburg, MD). Following incubation at 37° C for 30 minutes, the cDNA was ethanol precipitated in the presence of 2.5 M Ammonium Acetate, and electrophoresed on a 0.8% low melt agarose gel. The contaminating adapters and cDNA 2 kb or less in length were excised and discarded. The electrodes were reversed, and the cDNA was electrophoresed until the greater than 2 kb length cDNAs were concentrated near the lane origin. The areas of the gel containing the concentrated cDNAs were excised and placed in a microfuge tube, and the approximate volume of the gel slice was determined. An aliquot of water approximately three times the volume of the gel slice (300 µl) and 35 µl 10x β-agarose I buffer (New England Biolabs) was added to the tube, and the agarose was melted by heating to 65°C for 15 minutes. Following equilibration of the sample to 45°C, 3 µl of 1 U/µl β-agarose I (New England Biolabs, Beverly, MA) was added, and the mixture was incubated for 60 minutes at 45°C to digest the agarose. After incubation, 40 µl of 3 M Na acetate was added to the sample, and the mixture was incubated on ice for 15

minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose. The cDNA was ethanol precipitated, washed in 70% ethanol, air-dried and resuspended in 40 µl water.

Following recovery from low-melt agarose gel, The greater than 2 kb cDNA fractions were pooled and cloned into pBLUESCRIPT SK+ (Gibco/BRL Gaithersburg, MD)to yield the K562G library. The K562G library was created to enrich for receptor-encoding cDNAs, which are usually encoded by long cDNAs. Since long cDNAs normally constitute only a small fraction of the total cDNA species of the cell, the K562G library would also be enriched for cDNAs of low abundant mRNAs.

The resulting cDNA library were subjected to large scale sequencing to identify novel express sequence tags (ESTs). Sequencing was done using an ABI 377 sequencer using either the T3 or the reverse primer.

Example 2

Cloning of Zalpha27

Zalpha27 was discovered by the cDNA library of Example 1 with Expressed Sequence Tag of SEQ ID NO:3 the a clone was discovered and sequenced resulting in SEQ ID NOs: 1 and 2.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. A polypeptide comprised of an amino acid sequence selected from the group of SEQ ID NOs: 2 - 9.

2. An isolated polynucleotide that encodes a polypeptide comprised of an amino acid sequence selected from the group of SEQ ID NO: 2-9.

3. An antibody that specifically binds to a polypeptide selected from the group of SEQ ID NO. 2.

4. An educational kit comprised of a polynucleotide that encodes a polypeptide comprised of an amino acid sequence of SEQ ID NO. 3.

SEQUENCE LISTING

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acagaagtcg gtcctaggcc ccccaggctc tgaccttctt tcccagg atg agg tgg      176
                                                    Met Arg Trp
                                                     1

ggc cac cat ttg ccc agg gcc tct tgg ggc tct ggt ttt aga aga gca      224
Gly His His Leu Pro Arg Ala Ser Trp Gly Ser Gly Phe Arg Arg Ala
    5                  10                  15

ctc cag cga cca gat gat cgt atc ccc ttc ctg atc cac tgg agt tgg      272
Leu Gln Arg Pro Asp Asp Arg Ile Pro Phe Leu Ile His Trp Ser Trp
 20                 25                  30                  35

ccc ctt caa ggg gag cgt ccc ttt ggg ccc cct agg gcc ttt ata cgc      320
Pro Leu Gln Gly Glu Arg Pro Phe Gly Pro Pro Arg Ala Phe Ile Arg
                40                  45                  50

cac cac gga agc tcg gta gat agc gct ccc cca tcc ggg agg cat gga      368
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His His Gly Ser Ser Val Asp Ser Ala Pro Pro Ser Gly Arg His Gly
            55                  60                  65

cgg ctg ttc ccc agc gcc tct gca act gaa gct ata cag cgg cac cgc      416
Arg Leu Phe Pro Ser Ala Ser Ala Thr Glu Ala Ile Gln Arg His Arg
        70                  75                  80

cgg aac ctg gct gag tgg ttc agc cgg ctg ccc agg gag gag cgc cag      464
Arg Asn Leu Ala Glu Trp Phe Ser Arg Leu Pro Arg Glu Glu Arg Gln
    85                  90                  95

ttt ggc cca acc ttt gcc cta gac acg gtc cac gtt gac cct gtg atc      512
Phe Gly Pro Thr Phe Ala Leu Asp Thr Val His Val Asp Pro Val Ile
100                 105                 110                 115

cgc gag agt acc cct gat gag cta ctt cgc cca ccc gcg gag ctg gcc      560
Arg Glu Ser Thr Pro Asp Glu Leu Leu Arg Pro Pro Ala Glu Leu Ala
                120                 125                 130

ctg gag cat cag cca ccc cag gcc ggg ctc ccc cca ctg gcc ttg tct      608
Leu Glu His Gln Pro Pro Gln Ala Gly Leu Pro Pro Leu Ala Leu Ser
            135                 140                 145

cag ctc ttt aac ccg gat gcc tgt ggg cgc cgg gtg cag aca gtg gtg      656
Gln Leu Phe Asn Pro Asp Ala Cys Gly Arg Arg Val Gln Thr Val Val
        150                 155                 160

ctg tat ggg aca gtg ggc aca ggc aag agc acg ctg gtg cgc aag atg      704
Leu Tyr Gly Thr Val Gly Thr Gly Lys Ser Thr Leu Val Arg Lys Met
    165                 170                 175

gtt ctg gac tgg tgt tat ggg cgg ctg ccg gcc ttc gag ctg ctc atc      752
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ctg tgc caa ctt gtg gcc cag cgc tac acg ccc ctg aag gag gtt ctg      848
Leu Cys Gln Leu Val Ala Gln Arg Tyr Thr Pro Leu Lys Glu Val Leu
            215                 220                 225
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ccc ctg atg gct gct gct ggg tcc cac ctc ctc ttt gtg ctc cat ggc      896
Pro Leu Met Ala Ala Ala Gly Ser His Leu Leu Phe Val Leu His Gly
        230                 235                 240

tta gag cat ctc aac ctc gac ttc cgg ctg gca ggc acg gga ctt tgt      944
Leu Glu His Leu Asn Leu Asp Phe Arg Leu Ala Gly Thr Gly Leu Cys
    245                 250                 255

agt gac ccg gag gaa ccg cag gaa cca gct gct atc atc gtc aac ctg      992
Ser Asp Pro Glu Glu Pro Gln Glu Pro Ala Ala Ile Ile Val Asn Leu
260                 265                 270                 275

ctg cgc aaa tac atg ctg cct cag gcc agc att ctg gtg acc act cgg     1040
Leu Arg Lys Tyr Met Leu Pro Gln Ala Ser Ile Leu Val Thr Thr Arg
                280                 285                 290

ccc tct gcc att ggc cgt atc ccc agc aag tac gtg ggc cgc tat ggt     1088
Pro Ser Ala Ile Gly Arg Ile Pro Ser Lys Tyr Val Gly Arg Tyr Gly
            295                 300                 305

gag atc tgc ggt ttc tct gat acc aac ctg cag aag ctc tac ttc cag     1136
Glu Ile Cys Gly Phe Ser Asp Thr Asn Leu Gln Lys Leu Tyr Phe Gln
        310                 315                 320

ctc cgc ctc aac cag ccg tac tgc ggg tat gcc gtt ggc ggt tca ggt     1184
Leu Arg Leu Asn Gln Pro Tyr Cys Gly Tyr Ala Val Gly Gly Ser Gly
    325                 330                 335

gtc tct gcc aca cca gct cag cgt gac cac ctg gtg cag atg ctc tcc     1232
Val Ser Ala Thr Pro Ala Gln Arg Asp His Leu Val Gln Met Leu Ser
340                 345                 350                 355

cgg aac ctg gag ggg cac cac cag ata gcc gct gcc tgc ttc ctg ccg     1280
Arg Asn Leu Glu Gly His His Gln Ile Ala Ala Ala Cys Phe Leu Pro
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tcc tat tgc tgg ctc gtt tgt gcc acc ttg cac ttc ctg cat gcc ccc     1328
Ser Tyr Cys Trp Leu Val Cys Ala Thr Leu His Phe Leu His Ala Pro
            375                 380                 385

acg cct gct ggg cag acc ctt aca agc atc tat acc agc ttc ctg cgc     1376
Thr Pro Ala Gly Gln Thr Leu Thr Ser Ile Tyr Thr Ser Phe Leu Arg
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ctc aac ttc agt ggg gaa acc ctg gac agc act gac ccc tcc aat ttg     1424
Leu Asn Phe Ser Gly Glu Thr Leu Asp Ser Thr Asp Pro Ser Asn Leu
    405                 410                 415

tcc ctg atg gcc tat gca gcc cga acc atg ggc aag ttg gcc tat gag     1472
Ser Leu Met Ala Tyr Ala Ala Arg Thr Met Gly Lys Leu Ala Tyr Glu
420                 425                 430                 435

ggg gtg tcc tcc cgc aag acc tac ttc tct gaa gag gat gtc tgt ggc     1520
Gly Val Ser Ser Arg Lys Thr Tyr Phe Ser Glu Glu Asp Val Cys Gly
                440                 445                 450

tgc ctg gag gct ggc atc agg acg gag gag gag ttt cag ctg ctg cac     1568
Cys Leu Glu Ala Gly Ile Arg Thr Glu Glu Glu Phe Gln Leu Leu His
            455                 460                 465

atc ttc cgt cgg gat gcc ctg agg ttt ttc ctg gcc cca tgt gtg gag     1616
Ile Phe Arg Arg Asp Ala Leu Arg Phe Phe Leu Ala Pro Cys Val Glu
        470                 475                 480

cca ggg cgt gca ggc acc ttc gtg ttc acc gtg ccc gcc atg cag gaa     1664
Pro Gly Arg Ala Gly Thr Phe Val Phe Thr Val Pro Ala Met Gln Glu
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tac ctg gct gcc ctc tac att gtg ctg ggt ttg cgc aag acg acc ctg     1712
Tyr Leu Ala Ala Leu Tyr Ile Val Leu Gly Leu Arg Lys Thr Thr Leu
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caa aag gtg ggc aag gaa gtg gct gag ctc gtg ggc cgt gtt ggg gag     1760
Gln Lys Val Gly Lys Glu Val Ala Glu Leu Val Gly Arg Val Gly Glu
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gac gtc agc ctg gta ctg ggc atc atg gcc aag ctg ctg cct ctg cgg     1808
Asp Val Ser Leu Val Leu Gly Ile Met Ala Lys Leu Leu Pro Leu Arg
            535                 540                 545

gct ctg cct ctg ctc ttc aac ctg atc aag gtg gtt cca cga gtg ttt     1856
Ala Leu Pro Leu Leu Phe Asn Leu Ile Lys Val Val Pro Arg Val Phe
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Gly Arg Met Val Gly Lys Ser Arg Glu Ala Val Ala Gln Ala Met Val
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ctg gag atg ttt cga gag gag gac tac tac aac gat gat gtt ctg gac     1952
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580                 585                 590                 595

cag atg ggc gcc agt atc ctg ggc gtg gag ggc ccc cgg cgc cac cca     2000
Gln Met Gly Ala Ser Ile Leu Gly Val Glu Gly Pro Arg Arg His Pro
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gat gag ccc cct gag gat gaa gtc ttc gag ctc ttc ccc atg ttc atg     2048
Asp Glu Pro Pro Glu Asp Glu Val Phe Glu Leu Phe Pro Met Phe Met
            615                 620                 625

ggg ggg ctt ctc tct gcc cac aac cga gct gtg cta gct cag ctt ggc     2096
Gly Gly Leu Leu Ser Ala His Asn Arg Ala Val Leu Ala Gln Leu Gly
        630                 635                 640

tgc ccc atc aag aac ctg gat gcc ctg gag aat gcc cag gcc atc aag     2144
Cys Pro Ile Lys Asn Leu Asp Ala Leu Glu Asn Ala Gln Ala Ile Lys
    645                 650                 655

aag aag ctg ggc aag ctg ggc cgg cag gtg ctg ccc cca tca gag ctc     2192
Lys Lys Leu Gly Lys Leu Gly Arg Gln Val Leu Pro Pro Ser Glu Leu
660                 665                 670                 675

ctt gac cac ctc ttc ttc cac tat gag ttc cag aac cag cgc ttc tcc     2240
Leu Asp His Leu Phe Phe His Tyr Glu Phe Gln Asn Gln Arg Phe Ser
                680                 685                 690

gct gag gtg ctc agc tcc ctg cgt cag ctc aac ctg gca ggt gtg cgc     2288
Ala Glu Val Leu Ser Ser Leu Arg Gln Leu Asn Leu Ala Gly Val Arg
            695                 700                 705

atg aca cca gtc aag tgc aca gtg gtg gca gct gtg ctg ggc agc gga     2336
Met Thr Pro Val Lys Cys Thr Val Val Ala Ala Val Leu Gly Ser Gly
        710                 715                 720

agg cat gcc ctg gat gag gtg aac ttg gcc tcc tgc cag cta gat cct     2384
Arg His Ala Leu Asp Glu Val Asn Leu Ala Ser Cys Gln Leu Asp Pro
    725                 730                 735
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gct ggg ctg cgc aca ctc ctg cct gtc ttc ctg cgt gcc cgg aag ctg      2432
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740                 745                 750                 755

ggc ttg caa ctc aac agc ctg ggc cct gag gcc tgc aag gac ctc cga      2480
Gly Leu Gln Leu Asn Ser Leu Gly Pro Glu Ala Cys Lys Asp Leu Arg
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gac ctg ttg ctg cat gac cag tgc caa att acc aca ctg cgg ctg tcc      2528

Asp Leu Leu Leu His Asp Gln Cys Gln Ile Thr Thr Leu Arg Leu Ser
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aac aac ccg ctg acg gag gca ggt gtt gcc gtg cta atg gag ggg ctg      2576
Asn Asn Pro Leu Thr Glu Ala Gly Val Ala Val Leu Met Glu Gly Leu
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gca gga aac acc tca gtg acg cac ctg tcc ctg ctg cac acg ggc ctt      2624
Ala Gly Asn Thr Ser Val Thr His Leu Ser Leu Leu His Thr Gly Leu
    805                 810                 815

ggg gac gaa ggc ctg gag ctg ctg gct gcc cag ctg gac cgc aac cgg      2672
Gly Asp Glu Gly Leu Glu Leu Leu Ala Ala Gln Leu Asp Arg Asn Arg
820                 825                 830                 835

cag ctg cag gag ctg aac gtg gcg tac aac ggt gct ggt gac aca gcg      2720
Gln Leu Gln Glu Leu Asn Val Ala Tyr Asn Gly Ala Gly Asp Thr Ala
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gcc ctg gcc ctg gcc aga gct gcc cgg gag cac cct tcc ctg gaa ctg      2768
Ala Leu Ala Leu Ala Arg Ala Ala Arg Glu His Pro Ser Leu Glu Leu
            855                 860                 865

cta cac ctc tac ttc aat gag ctg agc tca gag ggc cgc cag gtc ttg      2816
Leu His Leu Tyr Phe Asn Glu Leu Ser Ser Glu Gly Arg Gln Val Leu
        870                 875                 880

cga gac ttg ggg ggt gct gct gaa ggt ggt gcc cgg gtg gtg gtg tca      2864
Arg Asp Leu Gly Gly Ala Ala Glu Gly Gly Ala Arg Val Val Val Ser
    885                 890                 895

ctg aca gag ggg acg gcg gtg tca gaa tac tgg tca gtg atc ctc agt      2912
Leu Thr Glu Gly Thr Ala Val Ser Glu Tyr Trp Ser Val Ile Leu Ser
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gaa gtc cag cgg aac ctc aat agc tgg gat cgg gcc cgg gtt cag cga     2960
Glu Val Gln Arg Asn Leu Asn Ser Trp Asp Arg Ala Arg Val Gln Arg
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cac ctt gag ctc cta ctg cgg gat ctg gaa gat agc cgg ggt gcc acc     3008
His Leu Glu Leu Leu Leu Arg Asp Leu Glu Asp Ser Arg Gly Ala Thr
            935                 940                 945

ctt aat cct tgg cgc aag gcc cag ctg ctg cga gtg gag ggc gag gtc     3056

Leu Asn Pro Trp Arg Lys Ala Gln Leu Leu Arg Val Glu Gly Glu Val
        950                 955                 960

agg gcc ctc ctg gag cag ctg gga agc tct gga agc tgagacactg         3102
Arg Ala Leu Leu Glu Gln Leu Gly Ser Ser Gly Ser
    965                 970                 975

gcggcaggca cctagctatg tgaccactgg ccctaaacct tttccctctg tgtcctcctg   3162
gcttgcactg ctccctctag aaagattcct tcaggtctgg aggcagagga atgggcatag   3222
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Phe Ile Arg His His Gly Ser Ser Val Asp Ser Ala Pro Pro Ser Gly
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                85                  90                  95
Glu Arg Gln Phe Gly Pro Thr Phe Ala Leu Asp Thr Val His Val Asp
            100                 105                 110
Pro Val Ile Arg Glu Ser Thr Pro Asp Glu Leu Leu Arg Pro Pro Ala
        115                 120                 125
Glu Leu Ala Leu Glu His Gln Pro Pro Gln Ala Gly Leu Pro Pro Leu
    130                 135                 140
Ala Leu Ser Gln Leu Phe Asn Pro Asp Ala Cys Gly Arg Arg Val Gln
145                 150                 155                 160

Thr Val Val Leu Tyr Gly Thr Val Gly Thr Gly Lys Ser Thr Leu Val
                165                 170                 175
Arg Lys Met Val Leu Asp Trp Cys Tyr Gly Arg Leu Pro Ala Phe Glu
            180                 185                 190
Leu Leu Ile Pro Phe Ser Cys Glu Asp Leu Ser Ser Leu Gly Pro Ala
        195                 200                 205
Pro Ala Ser Leu Cys Gln Leu Val Ala Gln Arg Tyr Thr Pro Leu Lys
    210                 215                 220
Glu Val Leu Pro Leu Met Ala Ala Ala Gly Ser His Leu Leu Phe Val
225                 230                 235                 240
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                245                 250                 255
Gly Leu Cys Ser Asp Pro Glu Glu Pro Gln Glu Pro Ala Ala Ile Ile
            260                 265                 270
Val Asn Leu Leu Arg Lys Tyr Met Leu Pro Gln Ala Ser Ile Leu Val
        275                 280                 285
Thr Thr Arg Pro Ser Ala Ile Gly Arg Ile Pro Ser Lys Tyr Val Gly
    290                 295                 300
Arg Tyr Gly Glu Ile Cys Gly Phe Ser Asp Thr Asn Leu Gln Lys Leu
305                 310                 315                 320
Tyr Phe Gln Leu Arg Leu Asn Gln Pro Tyr Cys Gly Tyr Ala Val Gly
                325                 330                 335
Gly Ser Gly Val Ser Ala Thr Pro Ala Gln Arg Asp His Leu Val Gln
            340                 345                 350
Met Leu Ser Arg Asn Leu Glu Gly His His Gln Ile Ala Ala Ala Cys
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Phe Leu Pro Ser Tyr Cys Trp Leu Val Cys Ala Thr Leu His Phe Leu
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His Ala Pro Thr Pro Ala Gly Gln Thr Leu Thr Ser Ile Tyr Thr Ser
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Ala Tyr Glu Gly Val Ser Ser Arg Lys Thr Tyr Phe Ser Glu Glu Asp
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Val Cys Gly Cys Leu Glu Ala Gly Ile Arg Thr Glu Glu Glu Phe Gln
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465 470 475 480
Cys Val Glu Pro Gly Arg Ala Gly Thr Phe Val Phe Thr Val Pro Ala
485 490 495
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595 600 605
Arg His Pro Asp Glu Pro Pro Glu Asp Glu Val Phe Glu Leu Phe Pro
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625 630 635 640
Gln Leu Gly Cys Pro Ile Lys Asn Leu Asp Ala Leu Glu Asn Ala Gln
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705 710 715 720
Gly Ser Gly Arg His Ala Leu Asp Glu Val Asn Leu Ala Ser Cys Gln

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Asp Leu Arg Asp Leu Leu Leu His Asp Gln Cys Gln Ile Thr Thr Leu
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Arg Leu Ser Asn Asn Pro Leu Thr Glu Ala Gly Val Ala Val Leu Met
785                 790                 795                 800
Glu Gly Leu Ala Gly Asn Thr Ser Val Thr His Leu Ser Leu Leu His
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Thr Gly Leu Gly Asp Glu Gly Leu Glu Leu Leu Ala Ala Gln Leu Asp
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        835                 840                 845
Asp Thr Ala Ala Leu Ala Leu Ala Arg Ala Ala Arg Glu His Pro Ser
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Gln Val Leu Arg Asp Leu Gly Gly Ala Ala Glu Gly Gly Ala Arg Val
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Gly Ala Thr Leu Asn Pro Trp Arg Lys Ala Gln Leu Leu Arg Val Glu
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gccatcaaga agaagctggg naagctgggc cggcaggtgc tgcccccatc agagctcctt 180
gaccacctct tcttccacta tgagttccag aaccagcgct tctccgctga ggtgctcagc 240
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Ser Val Asp Ser Ala Pro Pro Ser Gly Arg His Gly Arg
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        35                  40                  45
Cys Glu Asp Leu Ser Ser Leu Gly Pro Ala Pro Ala Ser Leu Cys Gln
    50                  55                  60
Leu Val Ala Gln Arg Tyr Thr Pro Leu Lys Glu Val Leu Pro Leu Met
65                  70                  75                  80
Ala Ala Ala Gly Ser His Leu Leu Phe Val Leu His Gly Leu Glu His
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Tyr Phe Gln Leu Arg Leu Asn Gln Pro Tyr Cys Gly Tyr Ala Val Gly
        35                  40                  45
Gly Ser Gly Val Ser Ala Thr Pro Ala Gln Arg Asp His Leu Val Gln
    50                  55                  60
Met Leu Ser Arg Asn Leu Glu Gly His His Gln Ile Ala Ala Ala Cys
65                  70                  75                  80
Phe Leu Pro Ser Tyr Cys Trp Leu Val Cys Ala Thr Leu His Phe Leu
                85                  90                  95
His Ala Pro Thr Pro Ala Gly Gln Thr Leu Thr Ser Ile Tyr Thr Ser
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Phe Leu Arg Leu Asn Phe Ser Gly Glu Thr Leu Asp Ser Thr Asp Pro
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Ser Asn Leu Ser Leu Met Ala Tyr Ala Ala Arg Thr Met Gly Lys Leu
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50 55 60
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65 70 75 80
Val Phe Gly Arg Met Val Gly Lys Ser Arg Glu Ala Val Ala Gln Ala
85 90 95
Met Val Leu Glu Met Phe Arg Glu Glu Asp Tyr Tyr Asn Asp Asp Val
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50 55 60
Leu Arg Asp Leu Glu Asp Ser Arg Gly Ala Thr Leu Asn Pro Trp Arg
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/18849

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, EMBL, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EMBL; ID HSAC384, AC AC000384, 18 April 1997 (1997-04-18) EVANS G A ET AL.: "Homo sapiens chromosome 11 clone pDJ393o15, *** sequencing in progress ***, 8 unordered pieces" XP002152367 Note: 92.1 % nt sequence identity with SEQ ID NO:1 in 1078 bp overlap. the whole document — -/—	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 November 2000	20/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van de Kamp, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. Jonal Application No
PCT/US 00/18849

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EMBL; ID HSW21966, AC W21966, 9 May 1996 (1996-05-09) MACKE J ET AL.: "59c7 Human retina cDNA Tsp509I-cleaved sublibrary Homo sapiens cDNA not directional, mRNA sequence" XP002152368 Note: 85.4 % nt sequence identity with SEQ ID NO:1 in 717 nt overlap. the whole document	1-4
A	WO 98 06845 A (LIFE TECHNOLOGIES INC ;CHATTERJEE DEB K (US); SHANDILYA HARINI (US) 19 February 1998 (1998-02-19) the whole document	1-4
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